New Rapid DNA Test for *Fusarium* Race 4 in Cotton

Paul Clark
Agdia, Inc.
Elkhart, IN

Abstract

Agdia, Inc, Elkhart, IN., has developed a new DNA based test for the rapid detection of *Fusarium* Race 4 (FOV4) in cotton tissue. The AmplifyRP test is a positive DNA test that performs without thermo cycling and requires less time than conventional PCR methods. The test may be performed in the field or lab following Agdia protocol and can distinguish the Race 4 genotype from other genotypes. The AmplifyRP *Fusarium* Race 4 test will be available commercially in March 2013.

Significance

The cotton industry faces the threat of the spread of *Fusarium* Race 4 through seed, soil, mechanical and other factors. Once present in the soil, the pathogen continues to reside and affect susceptible plants indefinitely. Identification of this pathogen in the field is instrumental in providing a preventative tool for FOV4 management. The significance of the test is it allows for positive identification of *Fusarium* Race 4 and proper containment measures to be implemented in the field to minimize the spread of the pathogen to other areas, both within and beyond the field. This effort will help to minimize the transfer of the pathogen to other productions areas by positively identifying FOV4 which will result in restricting the harvesting of seed, avoiding mechanical transfer by equipment and reducing transfer by other methods by farmers and field operators.

Development

Agdia has developed a new DNA application for pathogen testing allowing for positive detection of *FOV4*. Results may be read from suspect tissue samples within 30 minutes of the start of the test using a visual confirmation on an ImmunoStrip. This process is called AmplifyRP© and is designed for the testing of plant tissue in cotton. The rapid AmplifyRP© test positively identifies FOV4 in the cotton plant using a method that is faster than PCR and real-time PCR methods based methods. These methods can take hours or days to perform. Agdia's AmplifyRP© takes 30 minutes. The test saves valuable time and can be managed by people without specific technical skills. The AmplifyRP© test offers greater sensitivity and specificity over other test methods. The AmplifyRP© test requires no DNA or RNA purification making it easier to administer. It can be read visually using an ImmunoStrip and results can be read in thirty minutes versus hours by PCR methods.

Requirements

The test may be run in the field or in the lab with the proper protocol in place by the operator. Running this test requires:

- a constant heat source maintaining the temperature at a constant 39 degrees Celsius;
- a lyophilized pellet which includes the enzymes, recombinase, DNA polymerase and other proteins;
- other materials include an extraction buffer, rehydration buffer and running buffer;
- some disposables needed for liquid transfer and containment;

Protocol

The test procedure can be accomplished by following these seven simple steps.

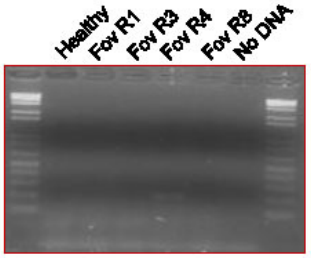
1. Place a small sample (size of a quarter) from a suspect plant into a sample bag between the mesh screens containing a proprietary extraction buffer.
2. Grind the sample using pen, knife handle or other hard object until slightly green in color, insuring extraction of plant DNA material.
3. Rehydrate the lyophilized pellet in micro centrifuge tube with 10ul of the rehydration buffer.
4. Transfer 1ul of the sample extract (#2 above) to the rehydrated pellet micro centrifuge tube (#3 above).
5. Place the micro tube containing extraction and rehydration mixture into the heat block for 15 minutes.

6. Transfer 1ul from micro tube in heat block (#5 above) to new micro tube and add 5 drops of running buffer then mix by swirling gently.
7. Place AmplifyRP© ImmunoStrip into this solution, wait five (5) minutes and interpret results visually on the ImmunoStrip.

Results

Agdia has shown consistent results in identifying *FOV4* against control and other genotypes of *Fusarium*. The following graphic depicts the comparison of testing methods using PCR and AmplifyRP© methods against healthy plants, *Fusarium* Race 1, 3, 4 and 8.

Detection of Fov R4 in cotton by both AmplifyRP nfo and PCR



An ethidium bromide-stained agarose gel showing the detection of Fov R4 in cotton by PCR

AmplifyRP strips showing the detection of Fov R4 in cotton by AmplifyRP nfo assay

Commercialization

Agdia intends to introduce for sale a commercial AmplifyRP© *FOV4* test kit to growers, field consultants, university and other affiliated organizations in March 2013. The test kits will be marketed in conjunction with and through an agreement with California Cotton Growers Association, available directly from Agdia. Agdia will accept orders, provide technical assistance or provide the test through their Testing Services Laboratory by calling 1-800-622-4342.